

(19)



Europäisches Patentamt

European Patent Office

Office européen des brevets



(11)

EP 0 841 067 B1

(12)

## EUROPEAN PATENT SPECIFICATION

(45) Date of publication and mention  
of the grant of the patent:  
**22.05.2002 Bulletin 2002/21**

(51) Int Cl.7: **A61K 39/395**

(21) Application number: **97120255.1**

(22) Date of filing: **28.02.1992**

### (54) **Freeze-dried preparation containing a monoclonal antibody**

Gefriergetrocknete Zubereitung, die einen monoklonalen Antikörper enthält

Préparation lyophilisée contenant un anticorps monoclonal

(84) Designated Contracting States:  
**CH DE DK FR GB IT LI NL SE**

- **Shimazaki, Yukio**  
**Mobara-shi, Chiba-ken 297 (JP)**
- **Takagi, Shiro**  
**Chosei-gun, Chiba-ken (JP)**

(30) Priority: **08.03.1991 JP 4343191**

(43) Date of publication of application:  
**13.05.1998 Bulletin 1998/20**

(74) Representative: **Harvey, David Gareth et al**  
**Graham Watt & Co.**  
**Riverhead**  
**Sevenoaks Kent TN13 2BN (GB)**

(62) Document number(s) of the earlier application(s) in  
accordance with Art. 76 EPC:  
**92906211.5 / 0 531 539**

(56) References cited:  
**EP-A- 0 177 836** **US-A- 2 149 304**

(73) Proprietor: **Mitsui Chemicals, Inc.**  
**Tokyo 100-6070 (JP)**

- **CHEMICAL ABSTRACTS, vol. 115, no. 7, 19**  
**August 1991 Columbus, Ohio, US; abstract no.**  
**68026, TAJIMA, MASAKAZU ET AL: "Method for**  
**preparation of freeze -dried agent for**  
**immunoagglutination test" XP002054970 & JP**  
**03 084 461 A (GREEN CROSS CORP.,**  
**JAPAN; TOKUYAMA SODA CO., LTD.;**  
**INTERNATIONAL REAGEN)**

(72) Inventors:

- **Fukuda, Tamotsu**  
**Mobara-shi, Chiba-ken (JP)**
- **Kuroiwa, Yasuyuki**  
**Hitachinaka-shi Ibaraki-ken (JP)**

EP 0 841 067 B1

Note: Within nine months from the publication of the mention of the grant of the European patent, any person may give notice to the European Patent Office of opposition to the European patent granted. Notice of opposition shall be filed in a written reasoned statement. It shall not be deemed to have been filed until the opposition fee has been paid. (Art. 99(1) European Patent Convention).

**Description**FIELD OF THE INVENTION

5 [0001] The present invention relates to a freeze-dried preparation comprising a monoclonal antibody(or antibodies) as a main ingredient(s).

PRIOR ART

10 [0002] A monoclonal antibody is a homogeneous globulin protein having reactivity to only a specific epitope. Recent progress in technologies of cell fusion, cultivation and protein purification, etc. has made it possible to produce large amounts of monoclonal antibodies. As a result, monoclonal antibodies have come to be utilized in various fields, such as various analyses, diagnoses, treatments and prophylaxes. In particular, expectations of monoclonal antibodies as medicines for treatments and prophylaxes are increasing. Above all, their application to human bodies is expected to be developed further in future, and development of human-derived monoclonal antibodies which are favorable in point of antigenicity is being advanced.

15 [0003] Hitherto, in this technical field, polyclonal antibodies such as immunoglobulin preparations have been used for the same purpose for medical diagnosis and treatment. While a monoclonal antibody is a homogeneous one having reactivity to only a specific epitope, a polyclonal antibody is a mixture of plural antibodies as is named so. Therefore, in a polyclonal antibody, plural molecules each having different properties act to mutually stabilize them so that such a polyclonal antibody is, as a whole, in a relatively stable state. However, in a purified monoclonal antibody, stabilization by the interaction between different molecules could not be expected so that such a monoclonal antibody is unstable to various physical and chemical actions irrespective of the immunoglobulin class of itself.

20 [0004] Globulin proteins such as monoclonal antibodies and polyclonal antibodies are often heated for the purpose of inactivating viruses therein, especially when they are used for medical diagnosis and treatment. Such globulin proteins are unsuitable to storage in solutions for a long period of time. Therefore, employment of freeze-drying formulation has been common for a stable storage of such globulin protein molecules. In addition, the globulin proteins are often treated with acids or alkali substances, if desired.

25 [0005] To such heat-treatment, freeze-drying and acid- or alkali-treatment, polyclonal antibodies are generally stable, but monoclonal antibodies would often be denatured and easily lose their activity by such treatment. In particular, IgM is less stable than any other monoclonal antibodies of other immunoglobulin classes (e.g., IgG, IgA and IgE). Regarding heat-treatment of antibodies, for example, JP-A 61-76423 (the term "JP-A" means an "unexamined published Japanese patent application") discloses the fact that monoclonal antibodies are unstable to heat-treatment and that, for the purpose of overcoming the thermal instability, a hydrolysate of ovalbumin is added to a monoclonal antibody preparation.

30 [0006] On the other hand, freeze-drying treatment involves a problem specific to monoclonal antibodies. Namely, in freeze-drying a monoclonal antibody, if a monoclonal antibody solution is freeze-dried without adding a stabilizer thereto, there occurs a problem of decrease of the antigen-binding activity of the monoclonal antibody due to denaturation of itself during freeze-drying. Therefore, it is necessary to prevent this problem. The problem is noticeable in freeze-drying a monoclonal antibody, while it is not so significant in freeze-drying a polyclonal antibody as a polyclonal antibody is stable because of the above-mentioned reasons.

35 [0007] In preparing a freeze-dried product of a monoclonal antibody, addition of albumin of a heterologous protein to a solution of a monoclonal antibody before freeze-drying (for example, JP-A60-146833, 61-78730 and 61-78731, and WO 90/11091) or addition of maltose of a saccharide thereto (for example, WO89/11297) is known.

40 [0008] Chemical Abstracts, Vol 115, No 7 in abstract No 68026 discloses a freeze-dried agent for immunoagglutination test, in which antibody-sensitized carrier particles are suspended in a solution to which albumin and/or dextran is added as a stabilizer. To the mixture, a compound selected from mannitol, sorbitol, inositol, citric acid and amino acid can be added before freeze drying.

45 [0009] Immunoglobulin preparations of polyclonal antibodies are usually used in a relatively high concentration, and aggregates would often form in the solutions during storage or during the succeeding freeze-drying treatment. The aggregates are considered to cause a serious anaphylactoid side effect, when the globulin containing them is intravenously injected to human bodies. Therefore, for the purpose of preventing formation of such aggregates, addition of a heterologous protein to the stock solutions is known. For instance, addition of gelatin alone of a heterologous protein to an immunoglobulin solution (for example, JP-A 58-167518, Vox. Sang. (1983) 51, 81-86) or addition of both sucrose of a saccharide and gelatin thereto is known to be effective for preventing formation of aggregates in the stock solutions and also for maintaining antibacterial and antiviral activities (SU 700132). All the technologies as disclosed above are to prevent formation of aggregates in a high concentration solution of an immunoglobulin of a polyclonal antibody. None of them mention or discuss the matter of decrease of the antigen-binding activity of polyclonal antibodies due to freeze-drying treatment. On the other hand, a monoclonal antibody is stored or freeze-dried in the form having a rel-

atively low concentration. Even under such a low concentration, however, there is still a problem of denaturation of a monoclonal antibody during freeze-drying as well as a problem of decrease of its antigen-binding activity. The matter has not heretofore been identified as to whether or not addition of gelatin used to prevent formation of aggregates of immunoglobulins would be useful for solving this problem.

[0010] On the other hand, it is widely known that carboxylic acids and their salts are used as a component of buffers for pH maintenance of various protein solutions. For instance, WO 89/11298 discloses addition of maltose, sodium chloride or sodium phosphate, as a stabilizer, to a stock solution of a monoclonal antibody for the purpose of preventing formation of aggregates to precipitate in the solution. It also mentions use of sodium citrate, instead of sodium phosphate, as a component of the buffer. However, it merely discloses a technology for preventing formation of aggregates in a stock solution of a monoclonal antibody during storage, and it does not disclose at all a treatment for freeze-drying a monoclonal antibody, a treatment for preventing denaturation of a monoclonal antibody during freeze-drying it and also a treatment for preventing decrease of the antigen-binding activity thereof. WO 89/11297 discloses a technology of adding maltose, as a stabilizer, to a monoclonal IgG antibody solution to be freeze-dried and further adding, as a buffer component, of 5 to 10 mM sodium acetate thereto so that the pH value of the solution is kept to fall within an acidic range of being from 3 to 6. In this case, sodium acetate is obviously used as a component of a buffer solution. WO 89/11297 suggests nothing as to the fact that carboxylic acid or its salt would still act as a stabilizer for preventing denaturation of antibody during freeze-drying treatment thereof in the pH range where the carboxylic acid or its salt does not have their buffer action. Regarding a pH range of an antibody solution, if an antibody solution having a low pH value is intravenously injected as an injection to a body, it would often cause a pain in the injected part. Where an antibody solution is used as an injection, it is desirable to have a pH value in an approximately neutral pH range. However, utilization of such a use of an antibody solution in a neutral pH range is not suggested in WO 89/11297.

[0011] For the purpose of inactivating viruses which would contaminate immunoglobulins in preparing them from sera or plasma, immunoglobulins are often heated in the form of their solutions. For instance, JP-A 62-292731, 61-194035, 61-191622 and 57-140724 disclose addition of carboxylic acids to said globulin solutions for this purpose. JP-A 61-78730 and 61-78731 disclose addition of sodium acetate to immunoglobulin preparations and heating them in a dry state. However, all of them merely mention addition of carboxylic acids for the purpose of stabilizing immunoglobulin preparations in the heat-treatment. It has not heretofore been known whether or not carboxylic acids and their salts would be useful for preventing denaturation of antibodies during freeze-drying of them and also for preventing decrease of an antigen-binding activity owing to said denaturation.

#### PROBLEM TO BE SOLVED BY THE INVENTION

[0012] The object of the present invention is to provide stable freeze-dried preparations of monoclonal antibodies, which are free from denaturation of the monoclonal antibodies during freeze-drying of them and from decrease of their antigen-binding activity owing to said denaturation.

#### MEANS FOR SOLVING THE PROBLEM

[0013] The present inventors earnestly studied for the purpose of solving the above-mentioned problem and, as a result, have found that gelatin, carboxylic acids or their salts, are effective for stabilizing a monoclonal antibody in freeze-drying it. Namely, they have found that, by addition of gelatin to a solution containing a monoclonal antibody to be freeze-dried, denaturation of the monoclonal antibody during freeze-drying it as well as decrease of its antigen-binding activity may be prevented. The use of gelatin for this purpose is the subject of the claims of parent application No 92 90 6211.5 (EP-B1-0 531 539). The inventors have also found that, by addition of carboxylic acid or its salt to a solution containing a monoclonal antibody to be freeze-dried, denaturation of the monoclonal antibody during freeze-drying it as well as decrease of its antigen-binding activity owing to said denaturation may be prevented in a broad pH range and even at a pH value being outside the range where a buffer action is exhibited. On the basis of this observation, they have found that it is possible to prepare a stable and highly safe preparation composition of a monoclonal antibody, and have completed the present invention.

[0014] According to the present invention, there is provided a preparation which is prepared by freeze-drying a solution comprising a monoclonal antibody and a carboxylic acid or a salt thereof acting as the sole stabilizer for said freeze-drying, and having pH of from 6.1 to 8.1.

[0015] Also according to the invention, there is provided use of a carboxylic acid or a salt thereof as a sole stabilizer for freeze-drying of a solution at a pH of from 6.1 to 8.1 containing a monoclonal antibody, said carboxylic acid or salt thereof stabilizing the antibody and preventing its denaturation and decrease of its antigen-binding activity upon freeze drying of the antibody.

[0016] Furthermore, the present invention provides a method for preventing a decrease of an antigen-binding activity of a monoclonal antibody owing to denaturation of the monoclonal antibody during freeze-drying a solution having pH

of from 6.1 to 8.1 and containing the monoclonal antibody by adding a carboxylic acid or its salt acting as the sole stabilizer for said freeze-drying to the solution.

[0017] Next, the present invention will be explained concretely hereunder.

[0018] The monoclonal antibody to be used in the present invention is any and every monoclonal antibody that is generally obtained from human beings, mice, rats and others, and the origins and the producing means are not specifically defined. For instance, the monoclonal antibody for use in the present invention may be obtained from a culture supernatant as obtained by cultivating antibody-producing cells as prepared by known methods such as cell fusion method or transformation method, or by cultivating cells into which a cloned antibody gene has been incorporated, or from ascites, etc of a mouse into which such antibody-producing cells have been transplanted. For purifying the monoclonal antibody obtained from such a cell culture supernatant or mouse ascites or the like, usable are various purification methods such as ammonium sulfate salting-out, ion exchange chromatography, gel filtration, affinity chromatography, ultra-centrifugation, adsorption chromatography and hydrophobic chromatography. The immunoglobulin classes of the monoclonal antibody to be used in the present invention are mostly IgG, IgM, IgA and IgE, but they are not specifically defined. A monoclonal antibody of any immunoglobulin class can be used in the present invention. Above all, IgM is less stable than those of other immunoglobulin classes. Therefore, the stabilizing method effective to IgM class monoclonal antibody may easily be applied to monoclonal antibodies of other immunoglobulin classes. In the present invention, a single monoclonal antibody may be used or plural monoclonal antibodies may be also used as a mixture of them with no problem.

[0019] As carboxylic acid, usable are, for example, citric acid, acetic acid, oxalic acid, succinic acid and fumaric acid. Citric acid is preferable. As salt of carboxylic acid, usable are, for example, sodium citrate, potassium citrate, sodium acetate, potassium acetate, sodium oxalate, potassium oxalate, sodium succinate, potassium succinate, sodium fumarate and potassium fumarate. Sodium citrate is preferable.

[0020] For purposes other than stabilizing the monoclonal antibody e.g. for pH adjusting, isotonicating and buffering the monoclonal antibody-containing solution to be freeze-dried, additives such as an inorganic salt may be further included, in addition to the carboxylic acid or its salt.

[0021] As inorganic salt, usable are, for example, sodium chloride, potassium chloride and magnesium chloride. Sodium chloride is preferable.

[0022] For producing the freeze-dried preparation of the present invention, a monoclonal antibody solution containing carboxylic acid or its salt may be freeze-dried. Preferably, a monoclonal antibody solution is added to a buffer containing carboxylic acid or its salt and having an adjusted pH value; or carboxylic acid or its salt is added to a monoclonal antibody-containing solution. The concentration of the monoclonal antibody in the solution to be used in the present invention is from 0.01 mg/ml to 50 mg/ml, preferably from 0.1 mg/ml to 10 mg/ml. The concentration of carboxylic acid or its salt to be added is from 2 mM to 500 mM, preferably from 10 mM to 200 mM.

[0023] The pH value of the solution of dissolving the monoclonal antibody to be freeze-dried is from 6.1 to 8.1, preferably from 6.5 to 7.8, when carboxylic acid is added. Adjustment of the pH value of the solution may be done by use of organic acids, inorganic acids, inorganic salts or other compounds which are generally used for pH adjustment, singly or in combination of two or more of them. As compound usable for such pH adjustment, there are mentioned, for example, citric acid, sodium citrate, potassium citrate, phosphoric acid, sodium phosphate, potassium phosphate, hydrochloric acid, tris(hydroxymethyl)aminomethane, acetic acid, sodium acetate, potassium acetate, sodium hydroxide, boric acid, sodium borate, and potassium borate. The concentration of the buffer of dissolving the monoclonal antibody is from 5 mM to 500 mM, preferably from 10 mM to 500 mM. As mentioned above, carboxylic acid or its salt may be also used for pH adjustment of a monoclonal antibody containing solution, and the above-mentioned amount of the acid or its salt indicates all the amount thereof in the solution including one for pH adjustment.

[0024] The thus prepared monoclonal antibody solution is well stable when freeze-dried directly as it is. It is also possible to add thereto a surfactant such as Tween 20 or Tween 80, a human or bovine albumin, or a chelating agent such as EDTA, for the purpose of isotonicating the solution or of preventing adhesion of the monoclonal antibody to the container containing the solution.

[0025] Freeze-drying of the monoclonal antibody solution may be carried out by any ordinary known method, and the drying temperature and the vacuum degree in the method may be selected suitably.

**EXAMPLES**

[0026] Next, the present invention will be explained by way of the following examples, which are, however, not limitative. IgM is exemplified herein as a monoclonal antibody in the present invention. This is because, as mentioned above, IgM is less stable than antibodies of other immuno-globulin classes (e.g. IgG, IgA and IgE), and therefore the stabilizing effect to be verified in IgM may be easily applied to antibodies of other immuno-globulin classes.

REFERENCE EXAMPLE:

[0027] This example teaches the use of gelatin to prevent denaturation of an antibody and decrease of its antigen binding ability. This use of gelatin is the subject of parent application No 92 90 6211.5 (EP-B-0 531 539).

[0028] Cells of Epstein-Barr virus (EB virus) transformed cell line MP-5038 (FERM BP-1596) producing an IgM antibody reactive to Group E serotype Pseudomonas aeruginosa were cultured, and a human monoclonal antibody was purified from the culture supernatant by ammonium sulfate salting-out, gel filtration with Sephadryl S-300 (Pharmacia Co.) and column chromatography with a hydroxyapatite HPLC column (Mitsui Chemicals, Inc) and Blue-sepharose (Pharmacia Co). The monoclonal antibody as obtained by these methods had a purity of 99% or higher, as analyzed by SDS-polyacrylamide gel electrophoresis and HPLC with a gel filtration column. The monoclonal antibody was dissolved in a phosphate-buffered physiological saline having an adjusted pH value of 7.4 (hereinafter referred to as PBS), to have a final concentration thereof of being 0.1 mg/ml. On the other hand, gelatin (high-grade gelatin; Nippi Co., type A (neutral gelatin) and type B (acidic gelatin)) was added thereto to have a final concentration thereof of being from 0.001 to 1%. The resulting solution was then put in 2 ml-volume polypropylene cryotubes (Corning Co.) under a sterilized condition, each in an amount of 0.5 ml, and frozen therein at -80°C. These were freeze-dried in vacuo. After being dried, the same amount, as that present before freeze-drying, of a distilled water for injection was added to the freeze-dried product so that the product was dissolved. The antigen-binding activity of the monoclonal antibody in the resulting solution was measured by the method mentioned below.

## 20 (Method of Measuring Antigen-Binding Activity)

[0029] Measurement of the antigen-binding activity of the anti-Pseudomonas aeruginosa antibody was carried in the manner mentioned below. A lipopolysaccharide (LPS), as prepared from formalin-killed cells of Group E serotype Pseudomonas aeruginosa ATCC 27581 by Tanabe et al's method (Menekijikkensousahou C, (1978) 1793-1801), was dissolved in PBS to have a concentration of 1 mg/ml, and this was diluted by 500-fold with 0.1 M phosphate buffer (pH 7.0). The thus diluted solution was then put in wells of a 96-well EIA plate (Immulon-600; Greiner Co.) in an amount of 50 µl/well. The plate was allowed to stand at 4°C overnight for coating, and it was then washed with PBS containing 0.05% Tween 20 (hereinafter referred to as a "washing solution"). A PBS containing 0.5% bovine serum albumin (hereinafter referred to as a "blocking solution") was added to each well in an amount of 200 µl/well, and the plate was then shaken at room temperature for one hour so that the non-specific protein-binding sites were saturated. After the blocking solution was removed, solutions of a sample to be tested, each having a multi-fold diluted concentration in order from a determined concentration, were put in the wells each in an amount of 100 µl/well, and the plate was then shaken for 2 hours at room temperature. After being washed with the washing solution four times, a peroxidase-labeled goat anti-human IgM antibody (Tago Co.) was diluted by 1000-fold with the blocking solution, and was put in each well in an amount of 100 µl/well, and the plate was shaken at room temperature for 2 hours. After being washed with the washing solution four times and then with 0.1 M citric acid buffer (pH 4.0) one time, a substrate solution containing 1 mg/ml of 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) and 0.003% hydrogen peroxide in the same buffer was put into each well in an amount of 50 µl/well, and the plate was then shaken at room temperature. After 30 minutes, 2% succinic acid was added to each well in an amount of 50 µl/well so that the enzymatic reaction therein was stopped. The absorbance at 414 nm was measured with a 96-well plate reader (Nippon InterMed Co.). Double logarithmic plotting was done between the reciprocal of the diluting magnification and the absorbance, and the diluting magnification to show the absorbance of being 0.1 was obtained and indicated as the antigen-binding activity of the sample tested.

[0030] The results are shown in Table 1, as a relative activity on the basis of the antigen-binding activity of the sample before frozen of being 10. Where a monoclonal antibody was freeze-dried without addition of gelatin thereto, the antigen-binding activity noticeably decreased. As opposed to the case, when gelatin was added, the antigen-binding activity was well recovered even in the freeze-dried product, and the effect depended upon the concentration of the gelatin added.

Table 1

Gelatin Concentration (%)	Antigen-Binding Activity	
	Neutral Gelatin	Acidic Gelatin
0	2	2
0.001	4	3
0.003	6	6
0.01	10	8
0.03	10	10

Table 1 (continued)

Gelatin Concentration (%)	Antigen-Binding Activity	
	Neutral Gelatin	Acidic Gelatin
0.1	10	10
1	10	10

Example 1:

[0031] 0.1 mg/ml, as a final concentration, of the same monoclonal antibody as that used in the Reference Example was dissolved in 20 mM phosphate buffer (pH 7) not containing or containing 2 or 10 mM sodium citrate, whereupon the salt concentration of the resulting solution was adjusted to be 150 mM with sodium chloride. The monoclonal antibody solution was then put in polypropylene cryotubes under a sterilized condition, each in an amount of 0.5 ml, and frozen therein at -80°C. These were freeze-dried in vacuo. The same amount, as that before freeze-drying, of a distilled water for injection was added to the freeze-dried product so that the product was dissolved. The antigen-binding activity of the monoclonal antibody in the resulting solution was measured.

[0032] The results in Table 2, are shown as a relative activity based on the activity of the sample before freeze drying of 10. Where the monoclonal antibody was freeze-dried in the absence of sodium citrate, the antigen-binding activity of the freeze-dried monoclonal antibody noticeably decreased. As opposed to the case, when the monoclonal antibody was freeze-dried in the presence of sodium citrate, then the antigen-binding activity of the freeze-dried monoclonal antibody was well recovered, depending upon the concentration of the sodium citrate as added.

Table 2

Sodium Citrate Concentration (mM)	0	2	10
Antigen-Binding Activity	2	5	9

Example 2:

[0033] 0.1 mg/ml, as a final concentration, of the same monoclonal antibody as that used in the Reference Example was dissolved in 50 mM phosphate buffer (pH 6.1 to 8.1) containing sodium citrate in a concentration of from 10 mM to 200 mM, whereupon sodium chloride was added thereto, if necessary, so that the salt concentration of the resulting solution became 150 mM. The resulting monoclonal antibody solution was then put in polypropylene cryotubes under a sterilized condition, each in an amount of 0.5 ml, and frozen therein at -80°C. These were freeze-dried in vacuo. The same amount, as that before freeze-drying, of a distilled water for injection was added to the freeze-dried product so that the product was dissolved. The antigen-binding activity of the monoclonal antibody in the resulting solution was measured.

[0034] The results obtained are shown in Table 3, as a relative activity based on the activity of the sample before frozen of 10. By adding sodium citrate at pH of from 6.1 to 8.1, the antigen-binding activity of all the freeze-dried products was well recovered.

Table 3

pH of Solution	Antigen-Binding Activity Sodium Citrate Concentration (mM)			
	10	50	100	200
6.1	10	10	10	10
7.0	9	10	10	10
8.1	9	10	10	-

Example 3:

[0035] 1 mg/ml, as a final concentration, of the same monoclonal antibody as that used in the Reference Example was dissolved in 0.1 M phosphate buffer (pH 7.0) containing sodium citrate (0.02 M), sodium chloride (0.05 M) and mannitol (0.5%). The resulting monoclonal antibody solution was then put in glass vials and frozen therein at -80°C. These were freeze-dried in vacuo. The same amount, as that before freeze-drying, of a distilled water for injection was added to the freeze-dried product so that the product was dissolved. The antigen-binding activity of the monoclonal antibody in the resulting solution was measured in the same manner as in the Reference Example. As a result, the

freeze-dried monoclonal antibody products were found to have the same antigen-binding activity as that of the samples before freeze drying.

Example 4:

**[0036]** Cells of human-human hybridoma MP5121 (FERM BP-2270) producing a human IgM reactive to Group A serotype Pseudomonas aeruginosa, which had been produced by cell fusion, were cultured, and the monoclonal antibody was purified from the culture supernatant in the same manner as in the Reference Example. The monoclonal antibody was dissolved in 0.1 M phosphate buffer (pH 7.0) containing sodium citrate (0.02 M), sodium chloride (0.05 M) and mannitol (0.5%), to have a final concentration thereof of being 1 mg/ml. The resulting monoclonal antibody solution was then put in glass vials and frozen therein at -80°C. These were freeze-dried in vacuo. The same amount, as that before freeze-drying, of a distilled water for injection was added to the freeze-dried product so that the product was dissolved. The antigen-binding activity of the monoclonal antibody in the resulting solution was measured in the same manner as in the Reference Example, provided that the antigen LPS was extracted from Group A serotype Pseudomonas aeruginosa (ATCC 27577). As a result, the freeze-dried monoclonal antibody products were found to have the same antigen-binding activity as that of the samples before freeze drying.

Example 5:

**[0037]** Monoclonal antibodies were purified from culture supernatants of cells of human IgM-producing human-human hybridoma MP5097, MP5139, MP5114 and MP5156 (PERM BP-2268, 2272, 2269 2339, respectively), all of which had been produced by cell fusion. These monoclonal antibodies had reactivity with Pseudomonas aeruginosa and were reactive to Groups B, E, G and I serotypes Pseudomonas aeruginosa, respectively. Five kinds of monoclonal antibodies comprising 4 kinds of these monoclonal antibodies and the monoclonal antibody used in Example 4 were dissolved in 0.1 M phosphate buffer (pH 7.0) containing sodium citrate (0.02 M), sodium chloride (0.05 M) and mannitol (0.5%), each in an amount, as a final concentration, of 5 mg/ml. These monoclonal antibody solutions were put in glass vials and frozen therein at -80°C. These were freeze-dried in vacuo. The same amount, as that before freeze-drying, of a distilled water for injection was added to each of the freeze-dried products so that each product was dissolved. The antigen-binding activity of each of the monoclonal antibodies in the resulting solutions was measured in the same manner as in the Reference Example, provided that as the antigen to each antibody, used were LPSs as extracted from ATCC 27577 (to Group A serotype), ATCC 27578 (to Group B serotype), ATCC 27581 (to Group E serotype), ATCC 27584 (to Group G serotype) and ATCC 27586 (to Group I serotype), respectively. As a result, the freeze-dried monoclonal antibody products were found to have the same antigen-binding activity to the five Pseudomonas aeruginosa LPSs of different serotypes, respectively, as that of the samples before freeze drying.

### ADVANTAGE OF THE INVENTION

**[0038]** In accordance with the present invention characterized by addition of carboxylic acid or its salt acting as the sole stabilizer for freeze drying, to a monoclonal antibody-containing solution to be freeze-dried, denaturation of a monoclonal antibody during freeze-drying can be well prevented so that a monoclonal antibody-containing freeze-dried preparation having a stable antigen-binding activity can be provided. The present invention may be applied to a monoclonal antibody of any immunoglobulin class, including IgG, IgM, IgA and IgE. Especially, it can be sufficiently applied to unstable IgM. The present invention may be well applied to any of human-derived, mouse-derived and rat-derived monoclonal antibodies. The number of the kinds of the monoclonal antibodies to be contained in the freeze-dried preparation of the present invention may be one or more.

**[0039]** The monoclonal antibody-containing freeze-dried preparation of the present invention may be used, like other immunoglobulin preparations, as an adjuvant for immunoauxotherapy for prophylaxis and treatment of bacterial infectious diseases and viral infectious diseases.

### **Claims**

1. A preparation which is prepared by freeze-drying a solution containing a monoclonal antibody and carboxylic acid or salt thereof acting as the sole stabilizer for said freeze-drying, and having pH of from 6.1 to 8.1.
2. The preparation as claimed in claim 1, in which the carboxylic acid is citric acid.
3. The preparation as claimed in claim 1 or claim 2, in which the monoclonal antibody is a human-derived one.

4. The preparation as claimed in any one of claims 1 to 3, in which the monoclonal antibody is one having an immuno-globulin class of IgM.

5. The preparation as claimed in any one of claims 1 to 4, in which the solution contains plural monoclonal antibodies.

6. Use of a carboxylic acid or a salt thereof as a sole stabilizer for freeze-drying of a solution at a pH of from 6.1 to 8.1 containing a monoclonal antibody, said carboxylic acid or salt thereof stabilizing the antibody and preventing its denaturation and decrease of its antigen-binding activity upon freeze drying of the antibody.

10. 7. The use as claimed in claim 6, in which the carboxylic acid is citric acid.

8. The use as claimed in claim 6 or claim 7, in which the monoclonal antibody is a human-derived one.

15. 9. The use as claimed in any one of claims 6 to 8, in which the monoclonal antibody is one having a globulin class of IgM.

10. 10. The use as claimed in any one of claims 6 to 9, in which the solution contains plural monoclonal antibodies.

20. 11. A method for preventing a decrease of an antigen-binding activity of a monoclonal antibody owing to denaturation of the monoclonal antibody during freeze-drying a solution having a pH of from 6.1 to 8.1 and containing the monoclonal antibody and by adding a carboxylic acid or a salt thereof as sole stabilizer for said freeze drying to the solution.

25. 12. The method as claimed in claim 11, in which the carboxylic acid is citric acid.

13. The method as claimed in claim 11 or claim 12, in which the monoclonal antibody is a human-derived one.

14. The method as claimed in any one of claims 11 to 13, in which the monoclonal antibody is one having a globulin class of IgM.

30. 15. The method as claimed in any one of claims 11 to 14, in which the solution contains plural monoclonal antibodies.

**Patentansprüche**

35. 1. Eine Zubereitung, welche hergestellt wird, durch Gefriertrocknung einer Lösung, enthaltend einen monoklonalen Antikörper und eine Karbonsäure oder ein Salz davon, die oder das als einziger Stabilisator für die besagte Gefriertrocknung dient, wobei der pH-Wert der Lösung zwischen 6,1 und 8,1 liegt.

40. 2. Die Zubereitung nach Anspruch 1, in der die Karbonsäure Zitronensäure ist.

3. Die Zubereitung nach Anspruch 1 oder nach Anspruch 2, in der der monoklonale Antikörper ein menschlicher ist.

45. 4. Die Zubereitung, nach einem der Ansprüche 1 bis 3, in der der monoklonale Antikörper zur Immunglobulin-Klasse IgM gehört.

5. Die Zubereitung, nach einem der Ansprüche 1 bis 4, in der die Lösung mehrere monoklonale Antikörper enthält.

50. 6. Verwendung einer Karbonsäure, oder eines Salzes davon, als einzigen Stabilisator für die Gefriertrocknung einer Lösung mit einem pH-Wert zwischen 6,1 und 8,1, enthaltend einen monoklonalen Antikörper, die besagte Karbonsäure oder ein Salz davon, die oder das den Antikörper stabilisiert und die Denaturierung sowie die Abnahme der Antigen-Bindungsaktivität während der Gefriertrocknung des Antikörpers verhindert.

55. 7. Die Verwendung nach Anspruch 6, in der die Karbonsäure Zitronensäure ist.

8. Die Verwendung nach Anspruch 6 oder nach Anspruch 7, in der der monoklonale Antikörper ein menschlicher ist.

9. Die Verwendung, nach einem der Ansprüche 6 bis 8, in der der monoklonale Antikörper zur Globulin-Klasse IgM

gehört.

**10.** Die Verwendung, nach einem der Ansprüche 6 bis 9, in der die Lösung mehrere monoklonale Antikörper enthält.

**5 11.** Ein Verfahren zur Verhinderung der Abnahme der Antigen-Bindungsaktivität eines monoklonalen Antikörpers infolge der Denaturierung des monoklonalen Antikörpers während der Gefriertrocknung einer Lösung, die einen pH-Wert zwischen 6,1 und 8,1 aufweist und den monoklonalen Antikörper enthält, und durch Zugabe zur Lösung einer Karbonsäure oder eines Salzes davon, als einzigen Stabilisator für die besagte Gefriertrocknung.

**10 12.** Das Verfahren nach Anspruch 11, in dem die Karbonsäure Zitronensäure ist.

**13.** Das Verfahren nach Anspruch 11 oder nach Anspruch 12, in dem der Antikörper ein menschlicher ist.

**15 14.** Das Verfahren nach einem der Ansprüche 11 bis 13, in dem der monoklonale Antikörper zur Globulin-Klasse IgM gehört.

**15 15.** Das Verfahren nach einem der Ansprüche 11 bis 14, in dem die Lösung mehrere monoklonale Antikörper enthält.

**20 Revendications**

**1.** Composition préparée par lyophilisation d'une solution contenant un anticorps monoclonal, et un acide carboxylique ou un sel de celui-ci agissant comme unique stabilisant pour ladite lyophilisation, et ayant un pH de 6,1 à 8,1.

**25 2.** Composition selon la revendication 1, dans laquelle l'acide carboxylique est l'acide citrique.

**3.** Composition selon la revendication 1 ou 2, dans laquelle l'anticorps monoclonal est un anticorps d'origine humaine.

**30 4.** Composition selon l'une quelconque des revendications 1 à 3, dans laquelle l'anticorps monoclonal est une immunoglobuline de la classe des IgM.

**5.** Composition selon l'une quelconque des revendications 1 à 4, dans laquelle la solution contient plusieurs anticorps monoclonaux.

**35 6.** Utilisation d'un acide carboxylique ou d'un sel de celui-ci comme unique stabilisant pour lyophiliser une solution ayant un pH de 6,1 à 8,1, contenant un anticorps monoclonal, ledit acide carboxylique ou son sel, stabilisant l'anticorps et prévenant sa dénaturation et la diminution de son activité de liaison à un antigène hors de la lyophilisation de l'anticorps.

**40 7.** Utilisation selon la revendication 6, dans laquelle l'acide carboxylique est l'acide citrique.

**8.** Utilisation selon la revendication 6 ou 7, dans laquelle l'anticorps monoclonal est un anticorps d'origine humaine.

**45 9.** Utilisation selon l'une quelconque des revendications 6 à 8, dans laquelle l'anticorps monoclonal est une globuline de la classe des IgM.

**10.** Utilisation selon l'une quelconque des revendications 6 à 9, dans laquelle la solution contient plusieurs anticorps monoclonaux.

**50 11.** Procédé de prévention d'une diminution de l'activité de liaison à un antigène d'un anticorps monoclonal due à la dénaturation de l'anticorps monoclonal au cours de la lyophilisation d'une solution ayant un pH de 6,1 à 8,1, et contenant l'anticorps monoclonal, et par addition d'un acide carboxylique ou d'un sel de celui-ci en tant qu'unique stabilisant de ladite lyophilisation de la solution.

**55 12.** Procédé selon la revendication 11, dans lequel l'acide carboxylique est l'acide citrique.

**13.** Procédé selon la revendication 11 ou 12, dans lequel l'anticorps monoclonal est un anticorps d'origine humaine.

**EP 0 841 067 B1**

14. Procédé selon l'une quelconque des revendications 11 à 13, dans lequel l'anticorps monoclonal en est une globulines de la classe des IgM.

5      15. Procédé selon l'une quelconque des revendications 11 à 14, dans lequel la solution contient plusieurs anticorps monoclonaux.

10

15

20

25

30

35

40

45

50

55